

# Grower-Adoptable Formulations of the Entomopathogenic Fungus *Metarhizium anisopliae* (Ascomycota: Hypocreales) for Sugarbeet Root Maggot (Diptera: Ulidiidae) Management

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**ABSTRACT** Producers in many North American sugarbeet (*Beta vulgaris* L.) growing areas rely heavily on organophosphate insecticides to manage the sugarbeet root maggot, *Tetanops myopaeformis* Röder. The threat of losing organophosphate options because of the potential for development of resistant root maggot strains or regulatory action has prompted a search for alternative control tools. American Type Culture Collection (ATCC) accession no. 62176, a strain of the entomopathogenic fungus *Metarhizium anisopliae* (Metschnikoff) Sorokin, was studied in field trials as a bioinsecticidal option for control of *T. myopaeformis* larvae because of shown virulence in preliminary laboratory testing. The fungus was evaluated at four field sites during 2001 and 2002 as a planting-time granule, an aqueous postemergence spray, or a combination of both. Three rates of *M. anisopliae* conidia,  $4 \times 10^{12}$  (1 $\times$ ),  $8 \times 10^{12}$  (2 $\times$ ), and  $1.6 \times 10^{13}$ /ha (4 $\times$ ) were applied as granules, and the spray was tested at the 1 $\times$  rate. A significant linear response in sucrose yield in relation to *M. anisopliae* granule application rate confirmed its entomopathogenic capacity under field conditions. Each multiple of *M. anisopliae* granules applied affected a yield increase of  $\approx 171$  kg sucrose/ha. The fungus was less effective than conventional insecticides at preventing stand loss from high root maggot infestations early in the season. It is concluded that, with additional research, mycoinsecticides could potentially be incorporated into management systems to complement chemical control tactics such as insecticidal seed treatments, soil insecticides (possibly at reduced rates), or postemergence materials for integrated control of *T. myopaeformis* adults or larvae.

**KEY WORDS** *Tetanops myopaeformis*, mycoinsecticide, bioinsecticide, insect-pathogenic fungus, entomogenous fungus

Few conventional chemical options are available to North American sugarbeet growers for managing the sugarbeet root maggot, *Tetanops myopaeformis* (Röder), a major insect pest of sugarbeet, *Beta vulgaris* L., in northern growing areas of the continent (Yun 1986, Cooke 1993, Campbell et al. 1998). Most options available for *T. myopaeformis* management during the past three decades have involved organophosphate and carbamate insecticides. Both classes have the same chemical mode of action (acetylcholinesterase inhibition) in insects. The profitability of sugarbeet

production in areas affected by this pest would be in jeopardy if an insecticide-resistant root maggot strain were to develop or, alternatively, if these insecticides became unavailable because of environmental concerns and regulatory action. These possibilities, along with an increasing consumer demand for reduced chemical pesticide use on food crops, stimulated a search for effective alternative control measures (Wozniak et al. 1993, Hodge et al. 1998, Campbell et al. 2000, Dregseth et al. 2003, Smigocki et al. 2003, Campbell 2005).

The entomopathogenic fungus *Metarhizium anisopliae* (Metschnikoff) Sorokin has been evaluated as a potential biological agent for managing insect pests in diverse crop and livestock production systems (Cilek et al. 1991, Samson et al. 1994, Kaaya and Munyinyi 1995, Kruger and Roberts 1997, Booth and Shanks 1998, Ekesi et al. 1998, Campbell et al. 2000). Environmental conditions that enhance the effectiveness of the fungus also have been documented (Walstad et al. 1970, Li and Holdom 1995).

Smith and Eide (1995) conducted bioassays on American Type Culture Collection (ATCC) accession no. 22099, a *M. anisopliae* strain from Israel. They

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observed 94% mortality in third-instar *T. myopaeformis* within 15 d after exposure to the fungus ( $2.3 \times 10^7$  conidia/ml), whereas 3% mortality occurred in non-exposed larvae. Based on those laboratory trials, ATCC 22099 was cultured on autoclaved whole-grain barley to produce inoculum for field testing. Data from field trials indicated that *M. anisopliae* was capable of reducing root maggot damage under low to moderate root maggot pressure (Campbell et al. 2000). In subsequent laboratory testing, Jonason et al. (2005) showed that ATCC 62176 was superior to ATCC 22099, several other *M. anisopliae* strains, and a *Beauveria bassiana* (Balsamo) Vuillemin isolate. However, information on the impacts of formulation, rate, or application timing on the pest management potential of *M. anisopliae* and, more specifically ATCC 62176, is lacking.

This study was designed to provide additional insight into the potential and limitations of *M. anisopliae* as a biological control agent for protecting sugarbeet from feeding injury by the sugarbeet root maggot. Furthermore, this study was carried out to determine if granular and aqueous sprayable formulations, applied by placement methods and equipment commonly used by producers for conventional chemical insecticide applications, could be used to effectively distribute infective units of the fungus for root maggot management.

### Materials and Methods

Field trials were established near Crookston, MN (Wheatville very fine sandy loam with 3.5% organic matter and 7.6 pH), and St. Thomas, ND (Bearded silt loam with 5.1% organic matter and 7.9 pH), in 2001, and near Crystal (Glyndon silt loam with 4.9% organic matter and 7.9 pH) and St. Thomas, ND (Glyndon silt loam with 3.6% organic matter and 7.9 pH), in 2002. Natural infestations of *T. myopaeformis* were relied on at all study sites for this experiment. Experimental units were six-row, 10.6-m-long plots with rows spaced 56 cm apart. The St. Thomas and Crookston trials were planted 11 and 22 May, respectively, in 2001. The 2002 trials were planted 28 and 29 May. All plots were thinned to  $\approx 76,500$  seedlings/ha before colonization of the field by *T. myopaeformis* adult flies. Weeds were controlled by using herbicides, cultivation, and hand weeding on an as-needed basis.

Fungus conidia were applied as a corn meal-based planting-time granule, a spray timed to coincide with peak adult fly activity, or a combination of the two. The *M. anisopliae* strain used (ATCC no. 62176) was originally isolated from soybean cyst nematode (*Heterodera glycines*, Ichinohe) in Illinois (Carris and Glawe 1989). It was reisolated on agar from infected *T. myopaeformis* larvae before spore production for field trials. The fungus was produced in a diphasic system (Bradley et al. 1992, 2002). Conidia from agar media were used to inoculate flasks of liquid medium (40 g/liter glucose, 10 g/liter  $\text{KNO}_3$ , 5 g/liter  $\text{KH}_2\text{PO}_4$ , 1 g/liter  $\text{MgSO}_4$ , 0.05 g/liter  $\text{CaCl}_2$ , and 2 g/liter yeast extract). Liquid cultures were incubated for 3–4 d at

$25 \pm 1^\circ\text{C}$  and 150 rpm. The prepared cultures were used to inoculate autoclaved (103 KPa for 20 min/kg) pearled barley (Minnesota Grain, Eagan, MN) in sterilized, vented, plastic mushroom spawn bags (Unicorn Implement and Manufacturing, Commerce, TX). Liquid cultures were hand-mixed with the substrate under aseptic conditions at a ratio of 1:2 (vol:wt), and the bags were heat-sealed. Solid substrate fermentation was conducted for 8 d at  $25 \pm 1^\circ\text{C}$  in constant darkness. Cultures were observed daily and crumbled by hand within spawn bags as needed to prevent binding and provide aeration throughout the culture substrate. Whole cultures were transferred to paper bags and dried for 7 d at  $24 \pm 1^\circ\text{C}$ . Conidia were harvested by mechanical sieving through 20- and 100-mesh sieves in an ultrasonic sieve shaker (AS200; Retsch, Newton, PA). Conidial fractions smaller than 100 mesh (0.15 mm) were retained.

The granular formulation consisted of *M. anisopliae* conidia bound to 16- to 20-mesh corn grit using 20% monosorbitan oleate (Tween 20; Sigma, St. Louis, MO) at 2% (vol:wt). Granules were prepared in 5-kg batches by first applying a coating of Tween binder to corn grit using an artist's air brush while manually mixing the carrier and then blending in a V-cone blender. The fungus was added to the carrier at  $3.6 \times 10^{11}$  viable conidia/kg, and the combination was blended to form a homogeneous mixture. Fungal sprays consisted of conidia suspended in 0.1% aqueous Tween 80 (Sigma). Sprays were applied as 13-cm bands centered over rows through three 4001E nozzles (one centered above and two directed at plant bases from each side of the row at  $45^\circ$  angles) at 2.8 kg/cm pressure, and incorporated into the top 1.5 cm of soil using 5-cm long rolling tines attached to a tractor-mounted toolbar.

The experiment was arranged in a randomized complete block design with four replications of the treatments at each site. Six *M. anisopliae* treatments were examined. Three rates of fungus granules,  $1\times = 4 \times 10^{12}$  (11.2 kg formulation/ha),  $2\times = 8 \times 10^{12}$  (22.4 kg/ha), and  $4\times = 1.6 \times 10^{13}$  conidia/ha (44.8 kg/ha), were applied using modified in-furrow (MIF) placement (Boetel et al. 2006). This technique involved dropping granules through a conventional planter-equipped in-furrow insecticide application tube directed over the seed furrow in a 5- to 8-cm band near the rear press wheel. The tube was oriented backward toward the rear press wheel to allow some soil to cover the seed before granules reached the furrow. This placement concentrated most of the material over the row. The  $2\times$  granular rate was also applied by using the "spoon," an alternative placement device consisting of an open-faced bander attached to the terminal end of the granular output tube and directed over the seed furrow (Boetel et al. 2006). The device is designed to laterally deflect the majority of granules into miniature concentrated swaths to the immediate outside edges of the furrow while depositing a small concentration of material inside the furrow as soil begins to cover the seed. Two  $1\times$  ( $4 \times 10^{12}$  conidia/ha) postemergence *M. anisopliae* spray treat-

ments also were evaluated in the experiment. One was applied as a stand-alone treatment, and the other was included in conjunction with a 1× planting-time (MIF) granular application of the fungus. A no-insecticide treatment and an intensive chemical regimen consisting of a planting-time MIF application of terbufos (Counter 15G [granular]; BASF, Research Triangle Park, NC) plus a postemergence band of chlorpyrifos (Lorsban 4E [emulsifiable concentrate]; DowAgroSciences, Indianapolis, IN) were included for comparative purposes. The terbufos and postemergence chlorpyrifos were applied at 1.8 and 0.37 kg (AI)/ha, respectively. All treatments were applied to the center four rows of each plot, and the outer two rows served as buffers between treatments.

Roots were assessed at each site for *T. myopaeformis* feeding injury after most larval feeding activity had ceased (late July or early August). Ten roots, randomly selected from the outer two treated rows of each plot (immediately adjacent to harvest rows), were hand dug, washed, and immediately evaluated in accordance with the rating scale of Campbell et al. (2000), in which 0 = no visible feeding injury and 9 = >75% of the root surface blackened by feeding scars. Ratings for individual plots were the mean of 10 roots. Stand loss was determined by dividing the difference between the number of seedlings present shortly before adult root maggot flight activity and the number of roots remaining at harvest by the number of original seedlings, and was expressed as a percentage. Harvest dates ranged from 21 September to 1 October. The two center rows of each plot were mechanically defoliated and harvested with a commercial two-row lifter modified to harvest experimental plots. All harvested roots were immediately weighed on site, and a randomly collected 10- to 12-root sample from each plot was sent to the American Crystal Sugar Co. Quality Tare Laboratory (East Grand Forks, MN) for sucrose and impurity (i.e., sodium, potassium, and amino-nitrogen) content determinations needed to calculate net recoverable sucrose yields. Recoverable sucrose is that portion of the total sucrose in the root that would be extracted under normal factory refining operations. Recoverable sucrose yield/ha is the product of kg recoverable sucrose/Mg and root yield, expressed as Mg/ha. Root yield and sucrose concentration were determined on a fresh-weight basis.

Environments (location by year combinations) were assumed to be random and treatments were considered fixed effects for the analysis of variance (ANOVA) (McIntosh 1983). Means were compared by using the Fisher protected least significant difference (LSD) test (Carmer and Walker 1985) at  $\alpha = 0.05$ . Additionally, single-degree contrasts were used to determine if response of dependent variables to *M. anisopliae* conidia application rate, applied MIF, was linear.

## Results

Both treatment ( $F = 4.64$ ;  $df = 7,21$ ;  $P < 0.001$ ) and environment ( $F = 238.55$ ;  $df = 3,12$ ;  $P = 0.028$ ) had

significant effects on recoverable sucrose yield (Table 1); however, interaction effects were not significant ( $F = 1.01$ ;  $df = 21,84$ ;  $P = 0.465$ ). Recoverable sucrose yields in *M. anisopliae* plots ranged from 3,432 kg/ha for the 1× MIF treatment at St. Thomas in 2002 to 9,561 kg/ha for the 4× MIF application at Crookston in 2001. At Crookston in 2001, the average sucrose yield (9,070 kg/ha) was 2.2 times the average sucrose yield at St. Thomas in 2002 (4,122 kg/ha), whereas intermediate yields of 5,196 and 5,147 kg/ha were observed at St. Thomas in 2001 and Crystal in 2002, respectively. Recoverable sucrose yield from 4× MIF plots (6,147 Mg/ha) was significantly greater than in 1× MIF *M. anisopliae* plots (5,584 Mg/ha) and no-insecticide controls (5,492 Mg/ha) when sucrose yields were averaged across environments. Although the 4× rate of *M. anisopliae* granules was the only fungal treatment that provided a significant improvement in sucrose yield over the no-insecticide control, a comparison of the 0, 1×, 2×, and 4× modified-in-furrow treatments indicated a significant linear increase ( $F = 8.26$ ;  $df = 1,21$ ;  $P = 0.005$ ) in sucrose yield in response to *M. anisopliae* application rate. Each multiple of fungus granules applied affected an increase in sucrose yield by  $\approx 171$  kg/ha (Fig. 1). Root yields followed similar patterns to sucrose yields with significant environment ( $F = 120.10$ ;  $df = 3,12$ ;  $P < 0.001$ ) and treatment ( $F = 3.81$ ;  $df = 7,21$ ;  $P = 0.008$ ) effects and no significant interaction effects ( $F = 1.49$ ;  $df = 21,84$ ;  $P = 0.102$ ). Environment means for root yields ranged from 33.7 Mg/ha at St. Thomas in 2002 to 58.7 Mg/ha at Crookston in 2001. Average root yield from the conventional insecticide regimen was 8.4 Mg/ha higher than from the no-insecticide controls and significantly better than all *M. anisopliae*-based treatments, which ranged from 40.8 to 42.9 Mg/ha. A small numerical (nonsignificant) increase in root yield seemed to be associated with increasing *M. anisopliae* application rate. In addition, when root yields were averaged across environments, the 4× *M. anisopliae* plots were 2.9 Mg/ha higher than the no-insecticide controls, although the difference was not statistically significant.

Similar to observations on the other yield components, environment had a significant ( $F = 24.81$ ;  $df = 3,12$ ;  $P < 0.001$ ) impact on sucrose concentration, although treatment differences ( $F = 1.98$ ;  $df = 7,21$ ;  $P = 0.107$ ) and treatment by environment interactions ( $F = 1.02$ ;  $df = 21,84$ ;  $P = 0.45$ ) were not detected.

Differences in root injury ratings were influenced by environment ( $F = 44.55$ ;  $df = 3,12$ ;  $P < 0.001$ ) and treatment ( $F = 4.52$ ;  $df = 7,21$ ;  $P = 0.003$ ), although interaction effects were not significant ( $F = 0.85$ ;  $df = 21,84$ ;  $P = 0.646$ ). Environment ( $F = 74.84$ ;  $df = 2,9$ ;  $P < 0.001$ ) and treatment ( $F = 5.67$ ;  $df = 7,14$ ;  $P = 0.003$ ) also influenced stand reduction. The only treatment that significantly reduced feeding injury to sugarbeet roots or increased plant survival was the intensive chemical insecticide regimen.

Table 1. Sugarbeet root maggot feeding injury and yield of sugarbeet treated with granular and liquid formulations containing *M. anisopliae* conidia, Crookston, MN, and St. Thomas, ND, 2001, and St. Thomas and Crystal, ND, 2002

Environment	Treatment								Environment mean <sup>a</sup>
	No insecticide	1× MIF	1× spray	1× MIF + 1× spray	2× MIF	2× spoon	4× MIF	Terbufos + chlorpyrifos	
Recoverable sucrose (kg/ha)									
Crookston 2001	8617	8990	9389	8904	8659	9147	9561	9303	9070a
St. Thomas 2001	5115	5202	4709	5344	4773	4686	5205	6540	5196b
Crystal 2002	4628	4710	4886	4954	5614	5244	5221	5917	5147b
St. Thomas 2002	3607	3432	3876	3891	4372	4193	4601	4992	4122c
Treatment mean	5492c	5584c	5715bc	5774bc	5855bc	5818bc	6147b	6688a	5884
Root yield (Mg/ha)									
Crookston 2001	57.4	58.0	60.8	57.5	58.6	58.7	59.4	58.9	58.7a
St. Thomas 2001	36.2	38.1	35.1	38.9	34.1	33.6	36.5	48.4	37.6b
Crystal 2002	35.4	37.9	39.0	35.7	42.4	38.1	38.0	46.3	39.1b
St. Thomas 2002	31.0	29.2	31.2	31.7	35.3	34.1	37.4	39.9	33.7c
Treatment mean	40.0b	40.8b	41.5b	41.0b	42.6b	41.1b	42.9b	48.4a	42.3
Sucrose (g/kg)									
Crookston 2001	163	166	166	166	160	167	172	169	166a
St. Thomas 2001	159	156	154	155	157	157	160	153	156b
Crystal 2002	148	142	143	155	150	153	153	145	149c
St. Thomas 2002	135	135	140	139	140	140	141	141	139d
Treatment mean	151a	150a	151a	154a	152a	154a	156a	152a	153
Root injury (0–9) <sup>b</sup>									
Crookston 2001	2.9	3.0	2.9	2.9	3.0	2.4	2.6	2.4	2.8c
St. Thomas 2001	6.1	5.8	5.4	5.1	5.6	5.6	5.5	4.3	5.4a
Crystal 2002	4.0	5.3	4.4	4.9	4.2	5.0	4.9	3.8	4.6b
St. Thomas 2002	5.2	4.8	4.8	4.8	4.3	5.1	5.0	3.6	4.7b
Treatment mean	4.5a	4.7a	4.4a	4.5a	4.3a	4.5a	4.5a	3.5b	4.4
Stand reduction (%)									
St. Thomas 2001	23.4	23.9	22.7	21.6	24.6	24.7	23.5	8.6	21.6a
Crystal 2002	34.7	31.8	35.8	38.9	23.4	26.6	24.5	10.5	28.3b
St. Thomas 2002	37.4	47.8	39.7	42.6	43.2	42.9	41.1	29.3	40.6c
Treatment mean	31.8a	34.5a	32.7a	34.4a	30.4a	31.4a	29.7a	16.3b	30.2

Treatment means within a row sharing a letter are not significantly different ( $P > 0.05$ ) based on LSD.

<sup>a</sup> Environment means followed by the same letter are not significantly different ( $P > 0.05$ ) based on LSD.

<sup>b</sup> Root injury rating: 0 = no feeding scars; 1 = 1–4 small scars; 2 = 5–10 small scars; 3 = up to 3 large scars or numerous small scars; 4 = a few large scars and/or numerous small scars; 5 = several large scars and/or heavy feeding on lateral roots; 6 = numerous scars, up to 25% of root blackened with *T. myopaeformis* feeding scars; 7 = 25–50% of root blackened by scars; 8 = 50–75% of root blackened; and 9 = >75% of root surface blackened (Campbell et al. 2000).

## Discussion

The 2001 crop at Crookston produced the highest root yields, sucrose concentrations, and consequently, the highest recoverable sucrose yields. Plots in the

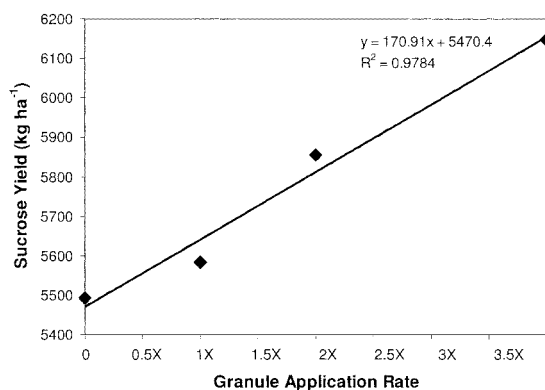


Fig. 1. Relationship of recoverable sucrose yield with *M. anisopliae* (strain ATCC 62176) granule application rate for *T. myopaeformis* management in sugarbeet. Fungus application rates were  $4 \times 10^{12}$  (1×),  $8 \times 10^{12}$  (2×), and  $1.6 \times 10^{13}$  (4×) conidia/ha.

Crookston environment also incurred the lowest levels of *T. myopaeformis* feeding injury. Treatment impacts on root yield, recoverable sucrose yield, and root injury at Crookston were small, but generally followed trends observed at the other sites. Recoverable sucrose yields at St. Thomas in 2002 averaged only 45% of those observed at Crookston in 2001. In all environments, the dual chemical insecticide treatment (terbufos 15G at planting plus chlorpyrifos 4E at peak adult fly activity) seemed to increase root yield and recoverable sucrose yield in comparison to the no-insecticide control plots. The greatest difference was observed at St. Thomas in 2001 where the conventional chemical insecticide program yielded 1,425 kg/ha more sucrose than the no-insecticide control. Despite the low root maggot feeding injury at Crookston, control plots produced 636 kg/ha less recoverable sucrose yield than those receiving the conventional chemical insecticide treatment. The average sucrose yield difference between plots receiving no insecticide and those treated with the dual chemical insecticide regimen was  $\approx 1,200$  kg/ha.

Root injury ratings provide validation that *T. myopaeformis* larvae were responsible for injury to plant roots and can give an indication of the relative severity



of larval feeding injury allowed by treatments. These ratings also provide evidence that at least a portion of yield differences observed among treatments and, to a lesser extent, environments can be attributable to the insect; however, visual differences in larval feeding injury are not always closely correlated with yield differences (Campbell et al. 1998). Feeding injury by *T. myopaeformis* larvae to the sugarbeet root system can impose a greater negative impact when the crop is subjected to other stressors (e.g., drought) than when growing conditions are more favorable (Campbell et al. 1998). The intensive dual insecticide program involving conventional chemical materials in our study did not entirely prevent larval feeding, yet it consistently resulted in the highest root and recoverable sucrose yields. Hence, most of the subsequent discussion emphasizes treatment effects on yield components.

The relatively small and frequently nonsignificant differences in sucrose concentrations within environments observed in this study indicate that the primary effect of *T. myopaeformis* feeding damage is on sugarbeet root yield. Differences in root yields between sugarbeet plots treated with conventional chemical insecticides and no-insecticide controls ranged from 1.5 Mg/ha at Crookston to 12.2 Mg/ha at St. Thomas in 2001. Yield differences among *M. anisopliae* conidia rates and application methods were generally small. Sucrose concentration and root yield tended to increase slightly with increasing fungus application rate when environment data were pooled, yet none of the fungus treatments were significantly better than the no-insecticide control with respect to these yield components; however, the subtle differences in sucrose concentration and root yield combined to produce significant treatment differences in recoverable sucrose yield ( $F = 4.64$ ;  $df = 7,21$ ;  $P = 0.003$ ). The  $4\times$  rate of *M. anisopliae* conidia produced  $655 \pm 247$  (SE) kg more sucrose per hectare than the no-insecticide control. This, and the linear increase in sucrose yield that corresponded with increased rates of *M. anisopliae*, provide strong evidence that the fungus is able to impose a detrimental effect on *T. myopaeformis* larvae under a variety of infestation levels and field conditions.

Fungus application rates used in this study were achieved by adjusting the application volume of granules containing the same concentration of conidia. The significant linear increase in sucrose yield with increasing application rate of the fungus suggests that applying higher volumes of granules or improving conidial distribution over that used in this study could increase the probability of larvae coming into contact with the fungus and thus, improve control of *T. myopaeformis*. Reducing granule size while maintaining the same output on a volumetric basis also could increase the likelihood of larval exposure because of the resulting increased number of fungus-coated granules deposited per unit soil volume into the target zone (i.e., plant base and rhizosphere). However, increasing output volume or applying smaller granules could pose logistical problems with application equipment

and, in turn, reduce the probability of producer adoption. Use of a liquid formulation at planting also could potentially overcome some of the concentration or conidial distribution problems associated with granules. Sugarbeet is especially vulnerable to the impacts of *T. myopaeformis* feeding injury when high infestations become established while plants are in the early seedling stages. Heavy feeding on small plants sometimes results in severing the taproot from the sugarbeet plant. This can cause plant mortality and subsequent yield loss because of poor stands. Stand reduction was not measured at Crookston but did not seem to be a factor in determining yields in that environment. In the three remaining environments, where *T. myopaeformis* larvae caused more severe root damage, stand losses ranged from 8.6% for the chemical insecticide treatment at St. Thomas in 2001 to 47.8% for the  $1\times$  *M. anisopliae* treatment at St. Thomas in 2002. Stand reductions in plots treated with the dual chemical insecticide treatment were approximately one half of those observed in fungus-treated plots. This pattern suggests that the time required for *M. anisopliae* to infect and kill larvae could be too long to prevent early feeding injury that causes seedling death, especially in years of early larval establishment on roots.

The average sucrose yield achieved in plots treated with the  $4\times$  rate of *M. anisopliae* granules and the significant linear sucrose yield response in relation to fungus application rate support previous laboratory research that demonstrated the entomopathogenic capability of *M. anisopliae* accession ATCC 62176 (Jonason et al. 2005). Yield differences in this study were not sufficient to allow differentiation between placement methods or application timings of the mycoinsecticide. It is apparent that conidia concentrations greater than the  $4\times$  rate used in this study or improved distribution of infective units within the sugarbeet seedling rhizosphere will be necessary for *T. myopaeformis* control in environments where damage potential and associated risks of yield loss are high. The strain of *M. anisopliae* used in this study was less effective than conventional chemical insecticides at preventing early season seedling losses, and is not likely to provide sufficient *T. myopaeformis* control in heavily infested areas when used as a stand-alone treatment, especially when high larval infestations occur early in the season. Integration with conventional chemical insecticides will likely be necessary if entomopathogenic fungi such as *M. anisopliae* are to be useful in areas where the sugarbeet root maggot is a consistent threat to sugarbeet production. Fungal bioinsecticides could potentially be incorporated into management programs to supplement chemical seed treatments, planting-time soil insecticides (possibly at reduced rates), or postemergence insecticides for *T. myopaeformis* adult and larval control. An important implication of this study is that the granular and sprayable formulations used for distribution of *M. anisopliae* conidia would be readily adoptable by sugarbeet producers because the materials can be applied by using commercially available equipment in a manner cur-

rently used for applying chemical insecticides. Because these formulations allow for more precise application of treatment rates than the barley inoculum used in previous research (Campbell et al. 2000), this trial provides important information on rate responses and some insights on application timing. Furthermore, the *M. anisopliae* strain used in this trial seemed to be more virulent to the root maggot than the strain (ATCC 22099) used in the earlier field trials of Campbell et al. (2000). That work and the research reported herein suggest that the greatest potential of *M. anisopliae* as a bioinsecticidal organism for *T. myopaeformis* management could be in areas where damage is usually slight to moderate.

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